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On studies of the microbial decomposition of various litters and humus formation in volcanic soils

INTRODUCTION

Many volcanic soils which contained much humic substance have been studied in the past two decades in Japan. It was well known that these volcanic soils generally showed high aluminium contents and great phosphorus fixing capacities were indicated. These aluminium compounds combining with humic substances inhibited the decomposition activities of soil micro-organisms so that the accumulation of humic substances in volcanic soils increased greatly.

In comparison with alluvial soils, these volcanic soils in Hokkaido Island contained much humic substance. Japanese oak (*Quercus dentata*), as one of the native plants in volcanic soils, is considered a basic material of these humic substances. Special characteristic zones were formed in southern parts of Hokkaido resulting from these undecomposable humic substances having accumulated in volcanic soils.

In spite of the presence of a large quantity of humic substances the potential soil fertility is extremely low. It is supposed that the causes of this low soil fertility are related to the physico-chemical properties of volcanic soils as maternal rocks. Furthermore, it is thought that the immobilisation of many nutritive materials by these humic substances indicates some morphological and physiological roles.

The purpose of this report was to indicate the decomposition process of Japanese oak litter in experimental and field conditions, and to estimate the relationship between the form of humic substances and the presence of Japanese oak litter in volcanic soils. Accordingly, the process of litter decomposition and the processes of humus formation which were related with microbial activities were investigated in this research.

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MATERIALS AND METHODS

The vegetation consisted of elm and sasa which are characteristic of humid meadow soils of lowland along margins of mountain slopes in Ishikari and alluvial soils distributed widely in the lowland of Ishikari Province. Vegetation consisting of oak and sasa are characteristic of volcanic soils forming on alluvial terraces or plateaus in Tokachi Province. Vegetation consisting of birch and sasa characterise heavy clay soils on coastal terraces or lowland in Kitami Province. Many species of *Sasa* are found as the predominant forest floor plants in Hokkaido Island.

The following were chosen for experimental materials: litters of birch *(Betula mandshurica),* sasa *(Sasa amphitricha),* oak *(Quercus dentata)* and elm *(Ulmus davidiana* var. *japonica),* and three types of soil: alluvial, volcanic and heavy clay. The litters were wintered under snow. They were collected after thawing in May, then were reduced to powder with a Wiley Mill and pH values were measured.

Changes in soil organic substances and humic substances and intensities of nitrification were tested. Each 25 g of soil and 75 g of litter was transferred to a separate Erlenmeyer flask (volume 1 litre) and was maintained at 60 % water holding capacity. The flasks were incubated at 30°C for 6 months after cotton plugging, and changes in organic components were analysed at intervals. In addition, 10 g samples of each litter were autoclaved in 300 ml flasks and were inoculated with *Penicillium* sp., *Coriolus hirsutus* and *Azotobacter vinelandii.* The organic components of the litters were then analysed. Waksman's method was used for analysis of soil organic substances; Springer's method for determination of humus morphology and Conway's method for analysis of ammonium and nitrate nitrogen. Another analysis was according to the manual for agrochemistry (Tokyo University).

For the purpose of bioassay of the growth inhibiting substances of microorganisms, three strains of bacteria, *Staphylococcus aureus, Bacillus subtilis* and *Escherichia coli* were used. These strains were obtained from the Type Culture Collection in the Department of Agricultural Chemistry, the University of Hokkaido, Sapporo.
The following three strains were applied to the litters: the wood-rotting fungus *Coriolus hirsutus,* isolated from Tokachi and Tomakomai Experimental Forest, the University of Hokkaido; *Penicillium* sp. isolated from

Nopporo National Forest (the Experimental Station for Silviculture) and *Azotobacter vinelandii* isolated from the Experimental Farm, the University of Hokkaido.

RESULTS AND DISCUSSION

The C/N ratio in soils and litters are given in Table 1.

Table 1: Carbon : Nitrogen ratio in soils and litters

	N%	C%	C/N
Birch (Betula)	1.05	72.00	68.5
Sasa (Sasa)	0.75	53.40	71.2
Oak (Quercus)	1.30	51.00	59.2
Elm (Ulmus)	1.60	42.00	26.2
Alluvial	0.23	3.60	15.6
Volcanic	0.05	1.20	24.0
Heavy Clay	0.17	2.10	12.3

The C/N ratios were high in the cases of *Betula, Sasa* and *Quercus*. On the other hand, the C/N ratio of *Ulmus* was low. The C/N ratios were high in heavy clay and alluvial soils and rather low in volcanic soils. Hayakawa (1958) recognised that the accumulation of humus was remarkable in volcanic soils. Tamura (1960) reported that the clear differences of humus morphology in soils of the same origin were the result of moisture conditions. In arid types of soils, *Quercus* and *Sasa* litter which accumulated on the soil surface commonly could not decompose. In contrast, in humid types of soils, the decomposition of litter proceeded and the humification of litter was accelerated.
Okada (1936) and Sasaki and Nakane (1943) indicated that the decomposition of various litters was related to soil micro-organisms. It was said that, in general, in arid types of soils fungi were predominant and in humid types of soils bacteria were predominant.
The decomposition of various litters is indicated in Table 2.

Four kinds of litters were tested during 6 months treatment. In this treatment three species of micro-organisms were inoculated separately. Changes in organic components were analysed after this inoculation at intervals of 3 and 6 months.

The lignin content of each of the three organic materials was about 50 %. Cellulose and hemi-cellulose contents were about 20% and 10% respectively. In contrast the contents of saccharides, proteins and resins were very low. Because the litters had been under snow during the winter it was thought that a large proportion of these components had decomposed on the soil surface during the previous autumn and the soluble fraction had been leached out in periods of thaw.

Table 2: Organic Components in Various Litters (%)

	months treated	resins	sugars	hemicel-luloses	cellu-loses	lignins	proteins
Treated with Penicillium sp.							
Birch	3	4,63	1.59	11.46	24.46	49.10	6.80
	6	2.63	1.37	11.28	22.34	49.06	1.28
Sasa	3	4.68	1.53	16.50	23.22	47.86	0.72
	6	2.73	1.13	16.30	19.29	43.74	2.28
Oak	3	7.82	1.47	13.68	17.12	49.10	0.64
	6	3.55	1.14	12.36	11.68	48.78	1.90
Elm	3	5.38	1.18	13.44	25.45	49.08	1.28
	6	2.79	1.01	11.28	19.13	48.02	1.96
Treated with Coriolus hirsutus.							
Birch	3	6.57	3.19	10.86	25.98	47.70	1.52
	6	2.43	0.31	10.68	23.04	47.48	1.24
Sasa	3	2.75	1.20	16.22	23.77	45.70	1.40
	6	2.67	0.51	15.18	22.70	45.10	2.80
Oak	3	4.83	2.35	12.48	24.05	48.80	1.92
	6	2.05	0.66	11.16	21.07	48.68	1.96
Elm	3	4.05	2.02	13.14	25.86	49.28	1.12
	6	1.15	0.81	10.62	22.91	47.84	1.20
Treated with Azotobacter vinelandii							
Birch	3	4.68	2.32	14.58	22.86	48.02	1.20
	6	3.98	1.12	10.92	20.50	47.74	2.08
Sasa	3	4.85	1.78	18.48	21.58	45.98	1.22
	6	3.39	1.49	14.88	19.89	44.22	2.24
Oak	3	5.24	3.84	13.62	21.34	48.82	1.36
	6	4.16	1.12	9.60	20.72	48.62	1.56
Elm	3	7.52	1.82	13.68	21.28	49.36	1.44
	6	2.33	1.07	11.28	18.80	49.14	2.28

It was considered that celluloses and proteins had been decomposed at considerable rates. There were no differences between the rates of decomposition of the lignins of the various litters. It was recognised that there was one distinct difference between celluloses and lignins. Considering various litters inoculated with *Coriolus hirsutus*, there was no difference in the amount of lignin decomposed after 3 months and after 6 months but there was some decomposition of cellulose between 3 and 6 months. After inoculation of micro-organisms, it was observed that decomposition of proteins, saccharides and celluloses occurred and especially that proteins and saccharides decomposed rapidly.

In Table 3, the results of analysis of the form of humus are given.

As a result of the decomposition of the litters by these micro-organisms, the colour coefficient of Japanese oak litter showed greater values than those of the other litters. On the other hand, the coefficient of *Sasa* showed the smallest values after treatment with *Penicillium* sp. It was clearly recognised that the degree of humification was greatest in *Sasa* and least in Japanese oak. The contents of humic acids were considerably increased as a whole.

When wood rotting fungi were inoculated into various litters, the degree of humification was the same as in litters inoculated with *Penicillium* sp. There were no differences on these points in the case of nitrogen fixing bacteria.

In these experiments, the humification of Japanese oak was especially slow and humic acids contents were comparatively high. Furthermore, the degree of humification was largest in alluvial soils, and smallest in volcanic soils.

The reason for the slow decomposition rates of Japanese oak in volcanic soils depends upon the high contents of tannin like substances. This relationship was observed in laboratory and field experiments.

For the determination of chemical activities of various litters, the evolution of carbon dioxide and the production of nitrate were measured in May and in August (Table 4). The activities of invertases and catalases were also investigated on the same materials (Table 5).

It was found that the decomposition of organic substances increased more in May than in August. These activities were considered to indicate the decomposition processes of organic substances.

Table 3: Humic Substances Produced from Various Litters

	months treated	EK_{S47}	EK_{S61}	F.Q.	Humid acid (mg/100 g)	Fulvic acid (mg/100 g)
Inoculated with Penicillium sp.						
Birch	3	0.730	0.222	3.28	47	24
	6	1.500	0.580	2.58	92	27
Sasa	3	0.583	0.190	3.06	48	11
	6	1.210	0.560	2.16	51	32
Oak	3	0.690	0.175	3.94	67	16
	6	1.990	0.560	3.55	158	41
Elm	3	0.315	0.095	3.31	63	15
	6	2.100	0.830	2.53	78	42
Inoculated with Coriolus hirsutus						
Birch	3	0.920	0.262	3.51	136	16
	6	2.000	0.880	2.27	150	111
Sasa	3	0.640	0.217	2.94	103	16
	6	1.500	0.550	2.72	109	87
Oak	3	0.960	0.245	3.91	110	19
	6	1.750	0.640	2.73	136	121
Elm	3	1.090	0.282	3.73	54	17
	6	1.320	0.415	3.18	134	44
Inoculated with Azotobacter vinelandii						
Birch	3	0.500	0.205	2.43	93	17
	6	1.900	0.780	2.43	107	79
Sasa	3	0.492	0.173	2.84	59	16
	6	1.200	0.455	2.63	123	33
Oak	3	1.090	0.282	3.86	116	25
	6	2.000	0.612	3.26	164	102
Elm	3	0.492	0.167	2.94	51	12
	6	1.200	0.395	3.04	111	50

In Table 6, the decomposition of litters by earthworms is indicated. The main conclusion was that celluloses and hemicelluloses were decomposed more than lignins.

CONCLUSIONS

The following conclusions were reached as the results of these experiments relating to the decomposition processes of various litters.

Table 4: Chemical activities of various litters

		CO_2 evolution ($mgCO_2$/100g)	NO_3 production ($mgNO_3$/100g)
Birch	May	15.08	2.89
	August	13.21	1.32
Sasa	May	14.76	2.62
	August	11.28	1.74
Oak	May	14.10	2.80
	August	11.38	1.30
Elm	May	13.20	2.77
	August	12.80	1.27

Table 5: Biochemical activities of various litters

		Invertases, mg sucrose/100g	Catalases, mgO_2/100g	H_2O %
Birch	May	383.3	183.1	24.7
	August	291.0	136.7	21.8
Sasa	May	313.3	175.5	23.7
	August	254.3	168.0	27.2
Oak	May	355.0	185.0	23.8
	August	362.0	158.7	24.4
Elm	May	220.0	182.4	26.0
	August	262.9	162.8	26.9

1. Growth inhibiting substances for micro-organisms were present in all litters tested. It was shown especially that the litter of Japanese oak contained these substances.
2. Among organic components of litters, the decomposition of saccharides, hemicelluloses, and celluloses was much greater, and of lignins was much smaller. It was clearly shown that *Ulmus* and *Quercus* decomposed faster than *Sasa* and *Betula*.
3. The humic substances which were formed from various litters were found more in the litters of *Ulmus* and *Quercus* than in the litters of *Sasa* and *Betula*.

Table 6: Organic components during treatments with earthworms (%)

		resins	saccha-rides	hemicel-luloses	cellu-loses	lignins	proteins
Birch	May	3.04	2.95	15.56	31.13	26.31	1.54
	August	2.54	2.85	14.56	31.03	21.29	2.25
Sasa	May	2.24	3.40	16.20	39.97	20.59	2.08
	August	2.43	2.80	15.27	36.23	19.08	2.93
Oak	May	2.40	4.88	10.83	15.71	42.21	2.90
	August	1.44	4.78	9.16	14.88	40.63	3.87
Elm	May	2.08	5.64	17.58	27.93	29.59	2.69
	August	1.27	5.12	12.94	24.74	28.23	2.73

4. The rate of decomposition of organic substances in alluvial soils was greater than in heavy clay soils. It was proved that in volcanic soils the decomposition of organic substances occurred slowly.
5. It was recognised that there were no great differences in nitrification between the soils. It was found that the production of nitrate was greater in alluvial soils than in heavy clay and greater in heavy clay than in volcanic soils. The production of nitrate was greater in litter of *Sasa* and *Betula* than in litter of *Ulmus* and *Quercus*.

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DISCUSSION

H.K. BHATTI: In Table 6, the decomposition of litter by earthworms is indicated. What species of earthworms were used?

S. TSURU: I have used only one species: *Eisenia foetida*

S.A. VISSER: Please inform us of the clay content and the clay type of your volcanic soil.

S. TSURU: The clay content was estimated at 7% by gravimetric measurement. Most of the clay was identified as hydrated-halloysite.

J. WEHRMANN: Is the high increase of protein content in Table 2 the result of a loss of organic material, or of a nitrogen fixation during incubation time?

S. TSURU: I cannot say positively, but I recognised the active processes of decomposition.

K. HAIDER: Did you find any decomposition of lignin by the fungi given in Table 2? The figures are relative ones, but what are the absolute values?

S. TSURU: I could not find any decomposition of lignin by fungi. These figures express percentages of the whole organic material.

I. SZABO: In your opinion, which methods are most useful for obtaining the litter extracts for the purpose of bioassay of the growth inhibiting substances?

S. TSURU: I used the following for extraction: deionized water, hot water (80° C), warm water (30° C), and cold water (4° C) using Seitz's filter. *Staphylococcus aureus, Bacillus subtilis* and *Escherichia coli* were used for the bioassay of these extracts. There were no clear distinctions between these treatments. It was concluded that the extracts were heat resistant and soluble.